

AD-A039 082

NAVAL BLOOD RESEARCH LAB BOSTON MASS
THE PHYSIOLOGICAL ROLE OF RED CELL 2,3 DPG IN OXYGEN TRANSPORT, (U)
AUG 73 C R VALERI

F/G 6/1

UNCLASSIFIED

NL

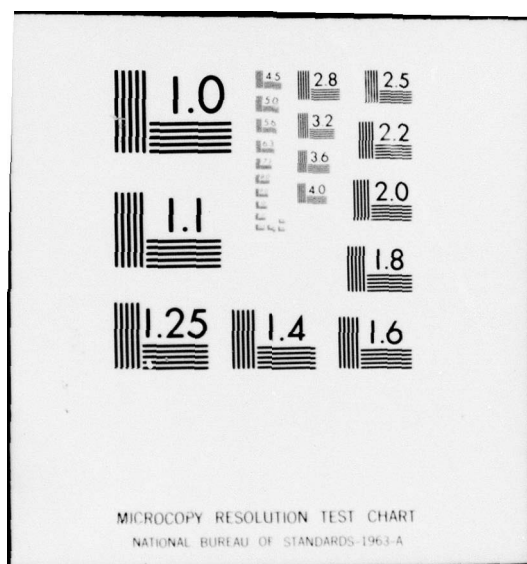
1 of 1
ADA039082



END

DATE
FILMED
5 - 77





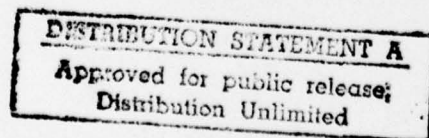
Sonderdruck aus:

ABHANDLUNGEN DER AKADEMIE
DER WISSENSCHAFTEN DER DDR

Jahrgang 1973

VII. Internationales Symposium über Struktur und Funktion der Erythrozyten

Veranstaltet von der Biochemischen Gesellschaft der DDR
in der Deutschen Gesellschaft für experimentelle Medizin und der
Akademie der Wissenschaften der DDR



ADA 039082

AD No. _____
DDC FILE COPY

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Naval Blood Research Laboratory 82 East Concord Street Boston, Massachusetts 02118	2a. REPORT SECURITY CLASSIFICATION Unclassified
	2b. GROUP

3. REPORT TITLE THE PHYSIOLOGICAL ROLE OF RED CELL 2,3 DPG IN OXYGEN TRANSPORT
--

4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Proceedings of the VIIth International Berlin Symposium on Structure and Function (Cont. 11

5. AUTHOR(S) (First name, middle initial, last name) CAPT C. Robert Valeri, MC, USNR
--

6. REPORT DATE 22-25 August 1973	7a. TOTAL NO. OF PAGES 11	7b. NO. OF REFS 20
--	-------------------------------------	------------------------------

8a. CONTRACT OR GRANT NO. Program Element: 63706N & 61151N	9a. ORIGINATOR'S REPORT NUMBER(S) 387 481
b. PROJECT NO. Task Area Number: MPN06.01 & MR041.02.01	
c. Work Unit Number: 0012 & 0017	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT No. 1	10a. DISTRIBUTION STATEMENT A Approved for public release; Distribution Unlimited
--	---

11. SUPPLEMENTARY NOTES of Erythrocytes, 22-25 Aug. 1973, Berlin, Akademie-Verlag, Berlin (DDR), pp 297-307.	12. SPONSORING MILITARY ACTIVITY Naval Medical Research & Development Command National Naval Medical Center Bethesda, Maryland 20014
--	--

13. ABSTRACT <p>The physiologic role of red cell 2,3 DPG has been studied in various situations. Healthy volunteers were studied after exposure to a simulated altitude of 4,500 meters. Stable anemic recipients were studied to determine the effects of therapeutic transfusions of red cells with low 2,3 DPG and high affinity for oxygen, and the effects of transfusion of red cells with 1-1/2 to 2 times normal 2,3 DPG levels. Hyper-ventilated, anemic baboons were studied to determine the effects of therapeutic transfusions of red cells with high 2,3 DPG and low affinity for oxygen and of red cells with low 2,3 DPG and high affinity for oxygen.</p> <p>The red cell 2,3 DPG level affects the in vivo P_{50} value which, in turn, affects erythropoietin production. In the baboon, the red cell 2,3 DPG level has been shown to increase oxygen delivery to tissue and decrease blood flow requirements to maintain oxygen consumption. In hyperventilated, anemic baboons, red cells with decreased 2,3 DPG and increased affinity for oxygen produced a significant increase in cerebral blood flow. The mechanism by which red cell 2,3 DPG affects cerebral blood flow is not known. When red cells with 1-1/2 to 2 times normal 2,3 DPG levels were transfused, the 2,3 DPG level in the circulation usually remained increased for 3 days after transfusion. Red cell 2,3 DPG is involved in oxygen transport by its effect on red cell production via erythropoietin production and by its effect on oxygen delivery to tissue via its ability to decrease red cell affinity for oxygen and to decrease blood flow.</p>

14

KEY WORDS

LINK A

LINK B

LINK C

ROLE

WT

ROLE

WT

ROLE

WT

Unclassified

THE PHYSIOLOGICAL ROLE OF RED CELL 2,3 DPG IN OXYGEN TRANSPORT

CAPT. C. Robert Vlahos, MC, USAF

15-58 August 1973

Program Element: 63706 A 61 51R

Task Area Number: 63706 A 61 51R

Work Unit Number: 63706 A 61 51R

of Cytochrome, 63-55 Aug. 1973, Berlin, Naval Medical Research & Development Command, Bethesda, Maryland 20814

The physiological role of red cell 2,3 DPG has been studied in various situations. Healthy volunteers were studied after exposure to a simulated altitude of 4,500 meters. Studies in patients were studied to determine the effects of therapeutic transfusions of red cells with 2,3 DPG and high affinity for oxygen, and the effects of transfusion of red cells with 1-1/2 to 2 times normal 2,3 DPG levels. Hyperventilation, anemia, and hypoxia were studied to determine the effects of therapeutic transfusions of red cells with high 2,3 DPG and low affinity for oxygen and of red cells with low 2,3 DPG and high affinity for oxygen.

The red cell 2,3 DPG level affects the in vivo P_{50} value which, in turn, affects erythrocyte production. In the baboon, the red cell 2,3 DPG level has been shown to increase oxygen delivery to tissues and decrease blood flow requirements to maintain oxygen consumption. In hyperventilated, anemic baboons, red cells with decreased 2,3 DPG and increased affinity for oxygen produced a significant increase in cerebral blood flow. The mechanism by which red cell 2,3 DPG affects cerebral blood flow is not known. When red cells with 1-1/2 to 2 times normal 2,3 DPG levels were transfused, the 2,3 DPG level in the circulation usually remained increased for 3 days after transfusion. Red cell 2,3 DPG is involved in oxygen transport by its effect on erythrocyte production and by its effect on oxygen delivery to tissues via its ability to decrease red cell affinity for oxygen and to decrease blood flow.

The Physiological Role of Red Cell 2,3 DPG in Oxygen Transport

By

C. R. VALERI

Oxygen transport is a complex biologic interrelationship involving ventilation, blood flow, red cell-hemoglobin mass, and red cell affinity for oxygen. Red cell affinity for oxygen *in vivo* is influenced by the character of the hemoglobin, the body temperature, the blood pH and P_{CO_2} , the carboxyhemoglobin and methemoglobin levels, and the red cell levels of 2,3 DPG, ATP, and inorganic phosphorus. Since the reports of BENESCH and BENESCH [1] and of CHANUTIN and CURNISH [2] indicating a direct relationship between the 2,3 DPG level and the hemoglobin affinity for oxygen, there has been considerable discussion of the physiologic importance of red cell 2,3 DPG in the preservation of red cells.

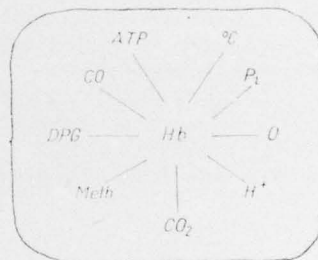


Fig. 1. Effects of H^+ , P_{CO_2} , carboxyhemoglobin, methemoglobin, temperature, red cell 2,3 DPG, ATP, and inorganic phosphorus on red cell-hemoglobin affinity for oxygen.

decreased affinity
for Oxygen

- 1) $\uparrow H^+$, $\downarrow pH$
- 2) $\uparrow pCO_2$
- 3) $\uparrow DPG$, $\uparrow ATP$, $\uparrow P_i$
- 4) $\uparrow Temp$

increased affinity
for Oxygen

- 1) $\downarrow H^+$, $\uparrow pH$
- 2) $\downarrow pCO_2$
- 3) $\downarrow DPG$, $\downarrow ATP$, $\downarrow P_i$
- 4) $\downarrow HbCO$
- 5) $\downarrow Meth Hb$
- 6) $\downarrow Temp$

This paper reports a series of observations made in our laboratory over the past 5 years regarding red cell 2,3 DPG and oxygen transport in man and in baboons. We have studied [1] the effect of red cell 2,3 DPG on affinity *in vivo* and the subsequent effect on erythropoietin production in normal male volunteers exposed to a simulated altitude of 4,500 meters; [2] the effects of red cells with low 2,3 DPG levels and increased affinity for oxygen, and red cells with elevated 2,3 DPG levels and decreased affinity for oxygen transfused to hyperventilated, anemic male baboons; [3] the effects of red cells with low 2,3 DPG levels and increased affinity for oxygen transfused to stable anemic recipients; and [4] the effects of therapeutic transfusion of red cells with 1-1/2 to 2 times normal, 2,3 DPG levels on red cell affinity for oxygen *in vivo* in stable anemic recipients.

The P_{50} value *in vivo* is a reflection of how the red cells are affected by pH, P_{CO_2} , temperature, red cell 2,3 DPG, ATP, and inorganic phosphate levels, and carboxyhemoglobin and methemoglobin levels (Fig. 1) [1-5]. The P_{50} value can be estimated by measuring the per cent saturation and the P_{O_2} in

A	BY	DISTRIBUTION/AVAILABILITY CODES	JUSTIFICATION	UNCLASSIFIED	NTIS	D.C.	White Section

each of two venous blood samples, or by measuring these in one venous blood sample and using an assumed slope ($n = 2.7$) of the oxyhemoglobin dissociation curve (Fig. 2). The P_{O_2} was measured at 37 C and the value was corrected to the temperature of the patient. A decrease in red cell affinity for oxygen is associated with increases in hydrogen ion concentration, temperature, P_{CO_2} , and red cell 2,3 DPG, ATP, and inorganic phosphate levels (Fig. 1). An increase in red cell affinity for oxygen is associated with decreases in hydrogen ion concentration, temperature, P_{CO_2} , and red cell 2,3 DPG, ATP, and inorganic phosphate levels, and with increases in carboxyhemoglobin and methemoglobin levels (Fig. 1).

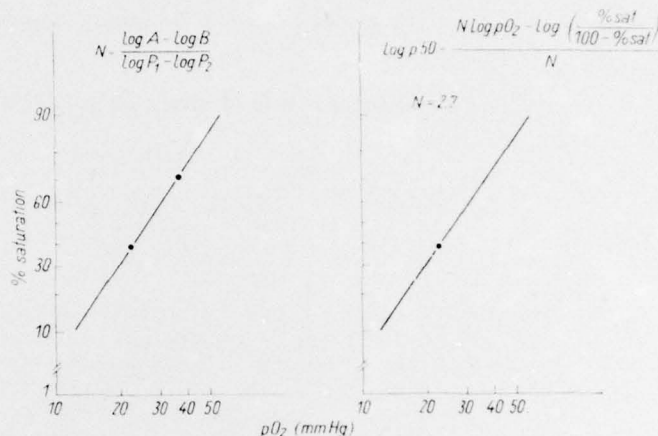


Fig. 2. Estimates of the *in vivo* P_{50} value from two venous blood samples and from one venous blood sample and an assumed slope of $n = 2.7$ of the oxyhemoglobin dissociation curve.

In the study of healthy volunteers exposed to a simulated altitude of 4,500 meters, the *in vivo* P_{50} value was estimated from venous blood obtained anaerobically from an antecubital blood sample without stasis while the subject was at rest. The *in vivo* P_{50} value was estimated from the per cent saturation and the P_{O_2} of the venous blood sample and an assumed slope of 2.7. During the 6 hours of exposure to simulated 4,500 meters the *in vivo* P_{50} decreased from 28.6 ± 1.0 mm Hg to 25.6 ± 1.2 mm Hg, at a time when the arterial P_{CO_2} decreased from 38 to 33 mm Hg, arterial P_{O_2} decreased to 70 mm Hg, and the arterial pH rose from 7.40 to 7.46 (Fig. 3) [6]. Arterial hypoxemia and alkalosis stimulate an increase in red cell 2,3 DPG during hypoxic exposure (Fig. 4) [7, 8, 9]. Lenfant and co-workers [7]

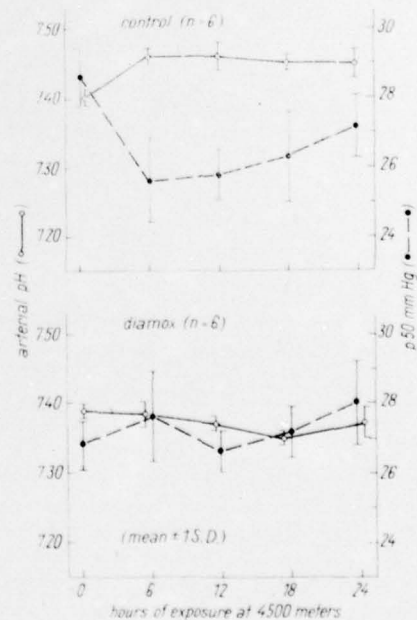


Fig. 3. Mean \pm standard deviation of arterialized capillary blood pH and *in vivo* P_{50} value in 6 healthy volunteers prior to and during 24 hours of exposure to simulated altitude of 4,500 meters. The upper panel shows the values during a control period, and the lower panel shows the values when 250 mg of Diamox (acetazolamide) was given every 8 hours beginning 24 hours prior to exposure (MILLER et al., 1973).

reported an increase followed by a decrease in urinary erythropoietin excretion in subjects exposed to high altitude. Miller and associates [6] reported that about 18 hours after exposure the erythropoietin level reached a maximum, and returned to normal within 24 hours of exposure (Fig. 5). They found that in both the serum and urine the initial increase and subsequent decrease correlated with the *in vivo* P_{50} value of the oxyhemoglobin dissociation curve. The erythropoietin-producing cells of the

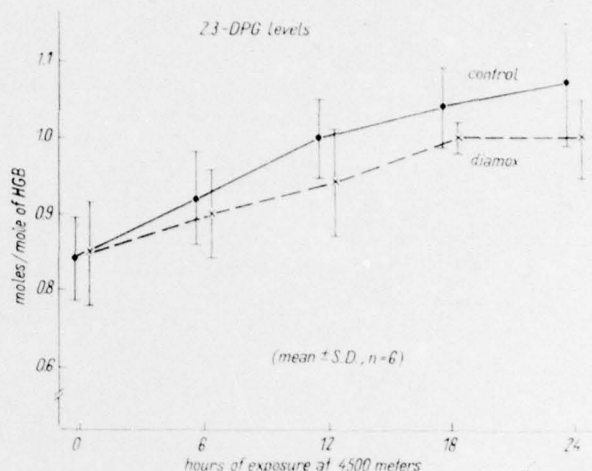


Fig. 4. Mean \pm standard deviation of red cell 2,3 DPG concentration in 6 volunteers after exposure to 4,500 meters of simulated altitude during a control period and after treatment with Diamox (MILLER et al., 1973).

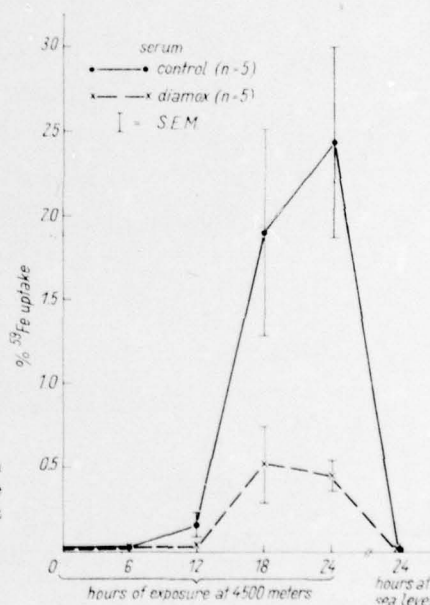


Fig. 5: Average serum erythropoietin concentration \pm 1 SEM in 5 volunteers after exposure to 4,500 meters of simulated altitude during a control period and after treatment with Diamox (MILLER et al., 1973).

kidney apparently monitor the per cent saturation of the blood in the venous portion of the micro-circulation. When the *in vivo* P_{50} value decreased, reflecting an increased red cell affinity for oxygen, erythropoietin was produced (Figs. 3, 5). The increased blood pH and low P_{CO_2} stimulated an increase in red cell 2,3 DPG which, in turn, decreased the red cell affinity for oxygen when the pH and P_{CO_2} were constant (Figs. 3, 4). The decrease in red cell affinity for oxygen as a result of the increased 2,3 DPG produced a decrease in erythropoietin production (Figs. 3, 4, 5). MILLER and associates [6] showed that treatment of the healthy volunteers with Diamox (250 mg 4 times a day) 24 hours prior to and after exposure to 4,500 meters prevented the alkalosis and the decrease in the *in vivo* P_{50} value, and blunted the erythropoietin increase in the serum and urine (Figs. 3, 5). They also found that treatment with Diamox significantly increased the red cell 2,3 DPG and serum inorganic phosphorus levels

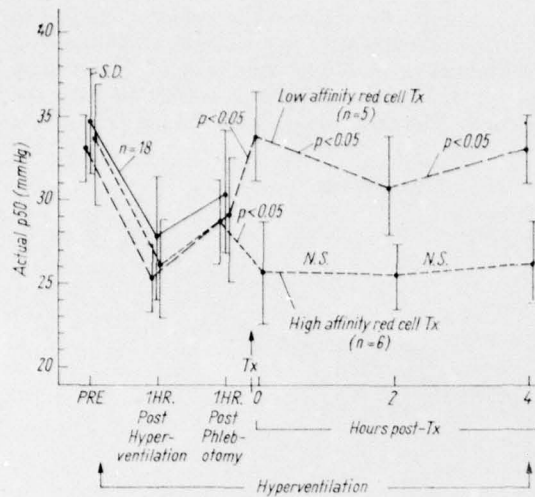


Fig. 6. Changes in actual P_{50} values prior to and 1 hour after hyperventilation, 1 hour after phlebotomy (2 hours after hyperventilation), and throughout the 4-hour period following the transfusion of red cells with high or low affinity for oxygen (VALERI et al., to be published).

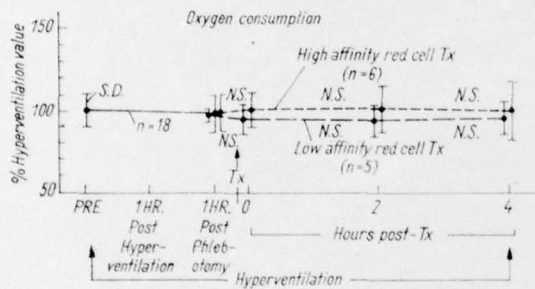


Fig. 7. Changes in oxygen consumption prior to and 1 hour after hyperventilation, 1 hour after phlebotomy (2 hours after hyperventilation), and throughout the 4-hour period following the transfusion of red cells with high or low affinity for oxygen (VALERI et al., to be published).

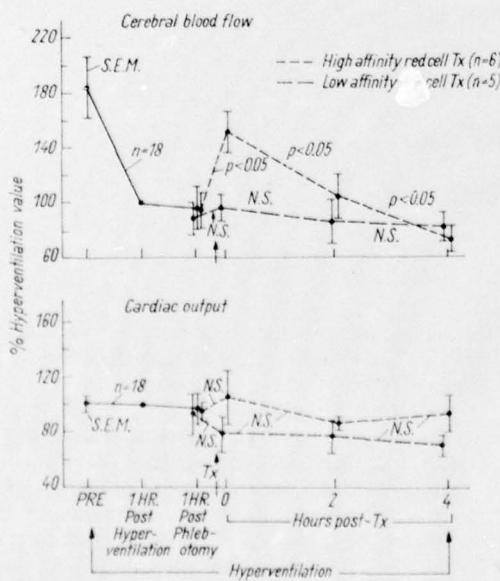


Fig. 8. Changes in cerebral blood flow and cardiac output 1 hour after phlebotomy (2 hours after hyperventilation) and throughout the 4-hour period following transfusion of red cells with high or low affinity for oxygen relative to the hyperventilated value (VALERI et al., to be published).

(Fig. 4) [6]. LENFANT and co-Workers [7], in a study similar to ours, found no increase in red cell 2,3 DPG. The increase observed in the study of MILLER and associates [6] was probably a result of the increase in plasma inorganic phosphorus.

Baboon red cells, like human red cells, undergo a loss of 2,3 DPG and an increase in affinity for oxygen during storage in the liquid anticoagulant citrate-phosphate-dextrose (CPD) [10]. Like human red cells, they can be rejuvenated with a solution containing pyruvate, inosine, glucose, phosphate, and adenine (PIGPA), a procedure which increases the red cell 2,3 DPG and ATP levels to about 1-1/2 times normal. The red cells are washed prior to transfusion to remove the potentially toxic substances used in the rejuvenation procedure [11]. The physiologic effects of transfusing red cells low in 2,3 DPG and with increased affinity for oxygen have never been clearly elucidated. We studied baboon red cells that were stored in CPD for 2 weeks and washed prior to transfusion (red cells low in 2,3 DPG and with increased affinity for oxygen) and red cells stored in CPD for 2 weeks, rejuvenated

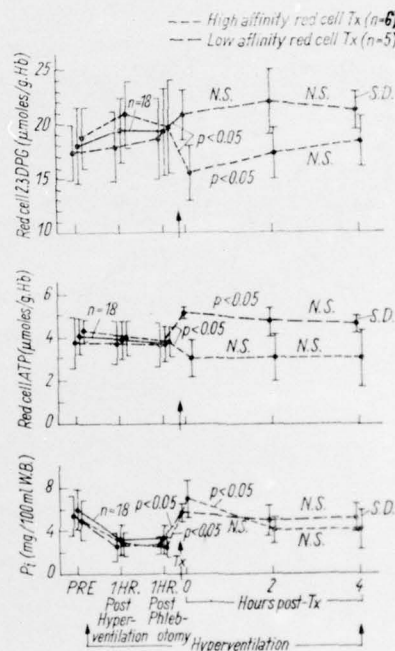


Fig. 9. Changes in red cell 2,3 DPG and ATP and blood inorganic phosphorus levels prior to and 1 hour after hyperventilation, 1 hour after phlebotomy (2 hours after hyperventilation), and throughout the 4-hour period following the transfusion of red cells with high or low affinity for oxygen.

with PIGPA, and washed prior to transfusion (red cells high in 2,3 DPG and with decreased affinity for oxygen) [12]. The baboons were anesthetized and passively hyperventilated by increasing the tidal volume and the respiratory rate. They remained hyperventilated throughout the study. After one hour of hyperventilation, an acute phlebotomy of approximately 500 ml of blood reduced the red cells by about 35%. Blood pressure and blood volume were maintained by infusing sodium chloride solutions in an amount 2 to 3 times the shed volume of blood. Red cell affinity for oxygen *in vivo* was estimated from a blood sample obtained from the pulmonary artery and one from the jugular vein, and the mean value is reported (Fig. 6). The *in vivo* P_{50} value was about 34 mm Hg prior to hyperventilation, and about 26 mm Hg after 1 hour of hyperventilation, at a time when the arterial P_{CO_2} was 20 mm Hg, and the pH was about 7.6 (Fig. 6). A decrease in the *in vivo* P_{50} from 34 to 26 mm Hg was associated with hyperventilation, but it produced no change in oxygen consumption, cardiac output, or cerebral blood flow (Figs. 6, 7, 8). The P_{O_2} decreased by about 10 mm Hg in the pulmonary artery, and by about 17 mm Hg in the jugular vein. The greater decrease in P_{O_2} in the jugular vein was associated with a decrease in cerebral blood flow. Hyperventilation was associated with a decrease in red cell and plasma inorganic phosphorus levels (Fig. 9). There was no change in oxygen consumption, cardiac output, or cerebral blood flow following the phlebotomy and infusion of crystalloid solutions (Figs. 7, 8).

Preserved red cells with 2,3 DPG levels of 3.0 μ M/g Hb, ATP levels of about 3.0 μ M/g Hb, and P_{50} values *in vitro* of about 24 mm Hg (measured by the BELLINGHAM and HUEHNS procedure [13], or red cells with 2,3 DPG levels of 18 μ M/g Hb, ATP levels of about 7.0 μ M/g Hb, and P_{50} values *in vitro* of about 41 mm Hg were rapidly infused into the hyperventilated, anemic baboons. The transfusion of red cells with low 2,3 DPG and high affinity for oxygen produced no significant change in oxygen

consumption, a slight but insignificant increase in cardiac output, and a highly significant increase in cerebral blood flow (Figs. 7, 8). The P_{50} value increased in both the pulmonary artery and the jugular venous blood samples. Immediately upon infusion there was a 3 mm Hg decrease in the *in vivo* P_{50} value and a decrease in the red cell 2,3 DPG level (Figs. 6, 9). During the 2-hour posttransfusion period the cerebral blood flow decreased to pre-transfusion levels, and this decrease was associated with a rapid increase in red cell 2,3 DPG (Figs. 8, 9) [14, 15]. The rapid increase in 2,3 DPG in baboons trans-

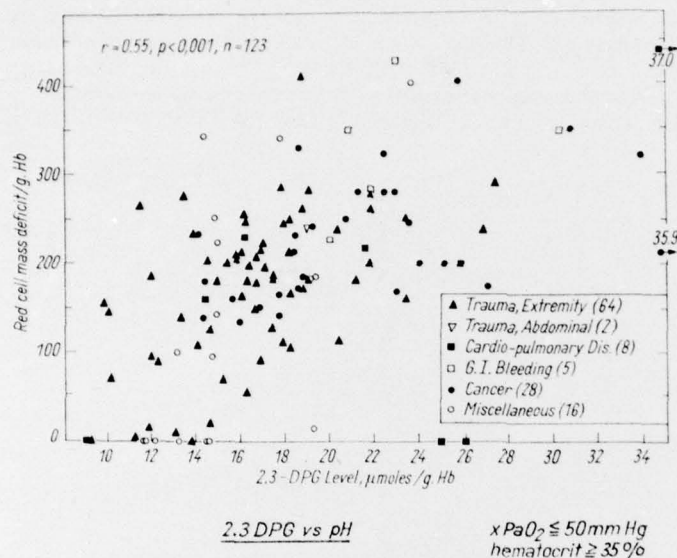


Fig. 10. Relation between red cell mass deficit and 2,3 DPG level (VALERI and FORTIER, 1969).

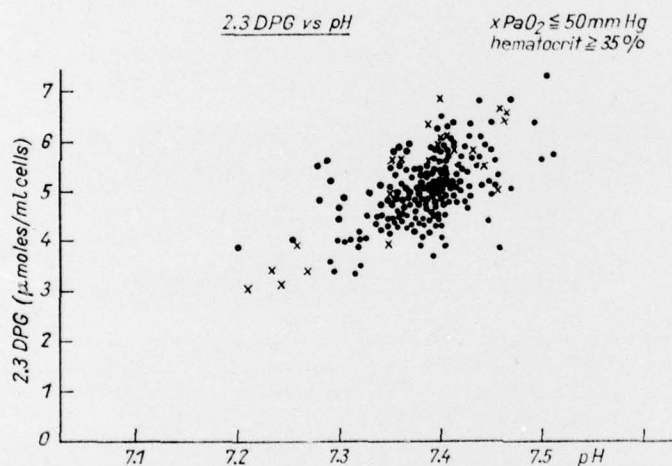


Fig. 11. 24-Hour posttransfusion survival and lifespan values of red cells stored in CPD at 4 C for 2 days prior to rejuvenation, and freeze-preservation with 40% W/V glycerol at -80 C. Three patients (R. W., M. L., J. W.) received the red cells within 4 hours of washing. The red cell 2,3 DPG and ATP levels and the P_{50} value are reported for each patient (VALERI, 1973).

fused with red cells low in 2,3 DPG was associated with an elevation in pH to about 7.6 and a decrease in the plasma inorganic phosphorus level. The *in vivo* P_{50} value did not increase during the 2-hour posttransfusion period, since with the increase in red cell 2,3 DPG there was a corresponding increase in blood pH. The changes in cerebral blood flow were not accompanied by changes in the *in vivo* P_{50} value (Figs. 6, 8). When the baboons received red cells with 2,3 DPG levels about 1-1/2 times normal and decreased affinity for oxygen, there was no change in oxygen consumption, and the decrease in the cerebral blood flow or cardiac output was insignificant (Figs. 7, 8). The transfusion of red cells with low affinity for oxygen increased the *in vivo* P_{50} value by 5 mm Hg (Fig. 6). The 2,3 DPG level increased during the 2-hour posttransfusion period, suggesting that there were substances in the rejuvenated red cells which were converted to 2,3 DPG after transfusion (Fig. 9). The P_{50} values were similar in the pulmonary artery and jugular venous blood whether the baboons received red cells with high or low 2,3 DPG levels. The auto-regulation of cerebral blood flow was apparently influenced by the 2,3 DPG level and not by the *in vivo* P_{50} value or the P_{50} level of the jugular vein (Figs. 6, 9). In baboons transfused with red cells with elevated 2,3 DPG levels, the red cell 2,3 DPG level decreased

to a normal value over the 24-hour posttransfusion period; in the baboon transfused with red cells with low 2,3 DPG levels, the red cell 2,3 DPG level increased to a normal value over the 24-hour posttransfusion period.

In another study, stable anemic recipients with slightly elevated blood pH were transfused with 3 to 5 units of washed, liquid-stored red cells low in 2,3 DPG and with increased affinity for oxygen [16]. The cardiac index increased immediately after the transfusion and it returned to normal within

Fig. 12. Effects of transfusing to F. W., a 20-year-old male with traumatic injuries, 6 units of rejuvenated, freeze-preserved, washed red cells with 2,3 DPG levels of 20 $\mu\text{M}/\text{g Hb}$ and P_{50} values of 38 mm Hg to correct a red cell mass deficit. Red cell mass, P_{50} value in vitro by the Bellingham and Huehns procedure, red cell 2,3 DPG and ATP levels, red cell pH, and plasma inorganic phosphorus levels are reported (VALERI, 1973).

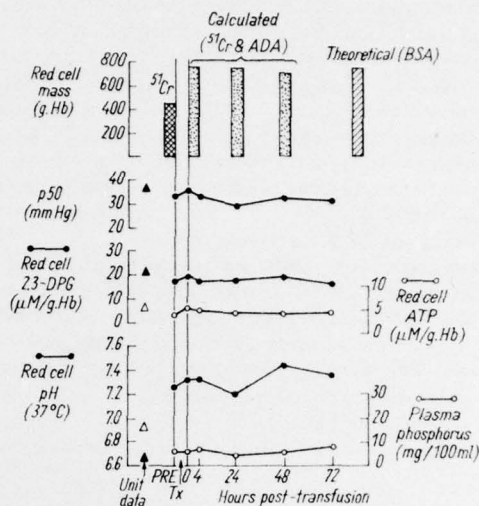
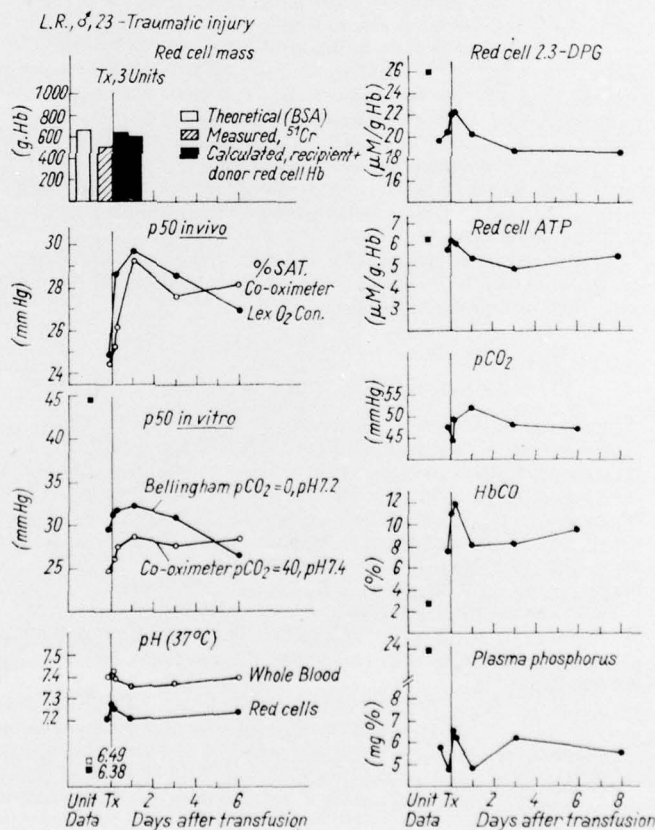


Fig. 13. Effects of transfusing to L. R., a 23-year-old male with traumatic injuries, 3 units of red cells with 2,3 DPG levels of 26 $\mu\text{M}/\text{g Hb}$ and *in vitro* P_{50} value of 45 mm Hg. Red cell mass, red cell P_{50} value *in vivo* and *in vitro*, whole blood and red cell pH, plasma inorganic phosphorus, red cell 2,3 DPG and ATP levels, and carboxyhemoglobin level are reported. Red cells were stored in ACD at 4 C for 2 days, rejuvenated, frozen with 40% W/V glycerol and store at -80°C , washed in the IBM Blood Cell Processor with 2.2 liters of sodium chloride solutions, and stored at 4 C for 24 hours in a sodium-chloride-glucose-phosphate solution (VALERI, 1973).



4 hours of the transfusion. The difference in oxygen content between the femoral artery and pulmonary artery decreased immediately after the transfusion, and within 4 hours after transfusion it was within normal limits. During the 4-hour posttransfusion period the increase in oxygen extraction from the systemic circulation was associated with an increase in red cell 2,3 DPG [16]. As in the baboon studies, the pulmonary artery P_{O_2} increased immediately after the transfusion. Circulating red cells with increased affinity for oxygen *in vivo* may pose a demand for increased blood flow or greater extraction of oxygen from the red cells, or both. Since it is difficult to assess P_{O_2} in tissue, mixed venous blood P_{O_2} is usually measured. This measurement reflects the combined effects of blood flow to tissue and the oxygen consumption by tissue, but it does not usually reflect the red cell affinity for oxygen.

The well-being of some patients may be placed in jeopardy if they are not able to meet the demand for increased cardiac output following transfusion of preserved red cells with high affinity for oxygen. Patients who require transfusion usually have elevated 2,3 DPG levels (Fig. 10) [17]. Thus, red cells with 1–1/2 to 2 times normal 2,3 DPG levels should be transfused to improve oxygen transport during the 24- to 72-hour posttransfusion period without demanding an increase in blood flow. Red cells can be stored in CPD at 4 C for 2 days before incubation with a PIGPA solution [11, 18–20]. After incubation at 37 C for 1 hour, red cells with elevated 2,3 DPG and ATP levels can be prepared. The red cells are then ready for glycerolization and freeze-preservation with either 40% W/V glycerol and storage at –80 C or with 20% W/V glycerol and storage at –150 C (Fig. 11). The 2,3 DPG levels of the transfused red cells will usually remain elevated for 2 to 3 days after infusion (Figs. 12, 13). Simple systems for washing previously frozen glycerolized red cells are now available for widespread clinical use. The washing procedures remove the adenine and inosine used in the rejuvenation solution. As many as 6 units of red cells with 1–1/2 to 2 times normal 2,3 DPG have been transfused to a single patient (Fig. 12).

Summary

The physiologic role of red cell 2,3 DPG has been studied in various situations. Healthy volunteers were studied after exposure to a simulated altitude of 4,500 meters. Stable anemic recipients were studied to determine the effects of therapeutic transfusions of red cells with low 2,3 DPG and high affinity for oxygen, and the effects of transfusion of red cells with 1–1/2 to 2 times normal 2,3 DPG levels. Hyperventilated, anemic baboons were studied to determine the effects of therapeutic transfusions of red cells with high 2,3 DPG and low affinity for oxygen and of red cells with low 2,3 DPG and high affinity for oxygen.

The red cell 2,3 DPG level affects the *in vivo* P_{50} value which, in turn, affects erythropoietin production. In the baboon, the red cell 2,3 DPG level has been shown to increase oxygen delivery to tissue and decrease blood flow requirements to maintain oxygen consumption. In hyperventilated, anemic baboons, red cells with decreased 2,3 DPG and increased affinity for oxygen produced a significant increase in cerebral blood flow. The mechanism by which red cell 2,3 DPG affects cerebral blood flow is not known. When red cells with 1–1/2 to 2 times normal 2,3 DPG levels were transfused, the 2,3 DPG level in the circulation usually remained increased for 3 days after transfusion. Red cell 2,3 DPG is involved in oxygen transport by its effect on red cell production via erythropoietin production and by its effect on oxygen delivery to tissue via its ability to decrease red cell affinity for oxygen and to decrease blood flow.

References

- [1] BENESCH, R., and R. E. BENESCH: *Biochem. Biophys. Res. Commun.* **26**, 162–167 (1967)
- [2] CHANUTIN, A., and R. R. CURNISH: *Arch. Biochem.* **121**, 96–102 (1967)
- [3] ASTRUP, P., M. RORTH and C. THORSHAUGE: *Scand. J. Clin. Lab. Invest.* **26**, 47–52 (1970)
- [4] ASTRUP, P.: *New Engl. J. Med.* **283**, 202–203 (1970)
- [5] GUEST, G. M., and S. RAPOPORT: *Am. J. Dis. Child.* **58**, 1072–1089, (1937)
- [6] MILLER, M. E., M. RORTH, H. H. PARVING, D. HOWARD, I. REDDINGTON, C. R. VALERI and F. STOHLMAN, Jr.: *New Engl. J. Med.* **288**, 706–710 (1973)
- [7] LENFANT, C., J. TORRANCE, E. ENGLISH, C. A. FINCH, C. REYNAFARJE, J. RAMOS and J. FAURA: *J. Clin. Invest.* **47**, 2652–2656 (1968)
- [8] LENFANT, C., J. D. TORRANCE and C. REYNAFARJE: *J. Appl. Physiol.* **30**, 625–631 (1971)
- [9] EATON, J. W., G. J. BREWER and R. F. GROVER: *J. Lab. Clin. Med.* **73**, 603–609 (1969)
- [10] HERMAN, C. M., F. L. RODKEY, C. R. VALERI and N. L. FORTIER: *Ann. Surg.* **174**, 734–743 (1971)
- [11] VALERI, C. R., and C. G. ZAROULIS: *New Engl. J. Med.* **287**, 1307–1313 (1972)
- [12] VALERI, C. R., M. RORTH, C. G. ZAROULIS, M. S. JAKUBOWSKI and S. V. VESCERA: To be published
- [13] BELLINGHAM, A. J., and E. R. HUEHNS: *Nature* **218**, 924–926 (1968)
- [14] VALERI, C. R., and N. M. HIRSCH: *J. Lab. Clin. Med.* **73**, 722–733, (1969)
- [15] KOPRIVA, C. J., J. L. RATLIFF, J. R. FLETCHER, N. L. FORTIER and C. R. VALERI: *Ann. Surg.* **176**, 585–589 (1972)

- [16] VALERI, C. R., and F. B. COLLINS: *J. Appl. Physiol.* **31**, 823–827, (1971)
 [17] VALERI, C. R., and N. L. FORTIER: *New Engl. J. Med.* **281**, 1452–1455 (1969)
 [18] DEUTICKE, B., J. DUHM and R. DIERKESMANN: *Pfluegers Arch.* **326**, 15–34 (1971)
 [19] OSKI, F. A., H. D. SUGERMAN and L. D. MILLER: *Blood* **30**, 522–525 (1972)
 [20] VALERI, C. R.: Presented at the 5th Annual Red Cross Scientific Symposium, May 7–8, 1973, Washington, D.C.

Discussion

RAPOPORT, S., Berlin (DDR):

1. Is it not likely that erythropoietin production is strongly affected by pH? The effect of Diamox to inhibit the erythropoietin production consists in a lowering of pH.

2. It would appear that the alkalosis with its effects on blood flow, inorganic P, and cellular metabolism, and the O_2 -dissociation curve have a complex importance. It may be that for such reasons the venous P_{O_2} tells us little what goes on.

3. I think that P_i is quite important, both for the DPG level and in its effects on tissue cells. You had some variations which are not clear to me.

VALERI, Chelsea:

Thank you Professor RAPOPORT for your stimulating questions. Regarding mixed venous P_{O_2} , our data suggest that this measurement is more a reflection of blood flow than of red cell affinity for oxygen. Our studies showed no relation between red cell affinity of preserved red cells and mixed venous P_{O_2} when adequate blood flow is maintained. In the baboon the P_{O_2} fell by 10 mm Hg in the pulmonary artery, and by 17 mm Hg after 1 hour of hyperventilation, during which time the red cell P_{50} value in vivo decreased by 7 mm Hg, cardiac output did not change, and cerebral blood flow significantly decreased. We have not found that the affinity for oxygen of preserved red cells influences the mixed venous P_{O_2} level when adequate blood flow is maintained.

Regarding your second question, it is possible that pH has a direct effect on erythropoietin production, and this action may have contributed to the reduced erythropoietin production in the Diamox-treated state. However, our data do not permit any conclusions in this respect.

In answer to your third question regarding the role of inorganic phosphate and factors influencing plasma phosphorus, the level of plasma inorganic phosphorus falls significantly with alkalosis and hypocarbia. Plasma inorganic phosphorus translocates from the blood into the tissue, and urinary phosphorus excretion is significantly reduced. As Dr. Rapoport noted, the plasma inorganic phosphate level increased immediately after transfusion because the washed red cells were suspended in a glucose-phosphate-sodium chloride solution (Fig. 9). As seen in Fig. 9, there was a significant decrease in the plasma inorganic phosphate level during the 2-hour posttransfusion period in the baboons who received red cells with low 2,3 DPG and increased affinity for oxygen. The decrease in plasma inorganic phosphorus was associated with the increase in the red cell 2,3 DPG level that occurred during this period. Understanding the control of plasma inorganic phosphorus metabolism is very important in understanding the complicated problem of oxygen transport.

MINAKAMI, Fukuoka:

We would like to present results of 2,3-diphosphoglycerate (DPG) analyses routinely carried out at National Nakano Chest Hospital for patients of thoracic diseases. The aim of this discussion is to analyze the relative contributions of three factors, namely, pH, PaO_2 and hematocrit values, on the DPG level.

Notable correlation was found between DPG and arterial pH as shown in Figure 1. This is in agreement with the *in vitro* observations. Similarly, negative correlation was found between DPG and hematocrit values in a group with physiological arterial pH, as shown in Figure 2. As acidotic and alkalotic cases were excluded, the often discussed contribution of hypoxic alkalosis may be neglected. Fig. 3 shows the dependence of the DPG level on PaO_2 in cases with physiological pH and hematocrit values. Although the increased level of DPG was observed in cases with low PaO_2 , relatively poor correlation was found.

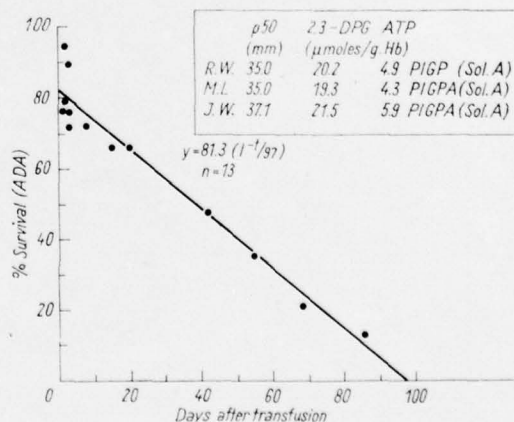


Fig. 1. The correlation between pH and DPG

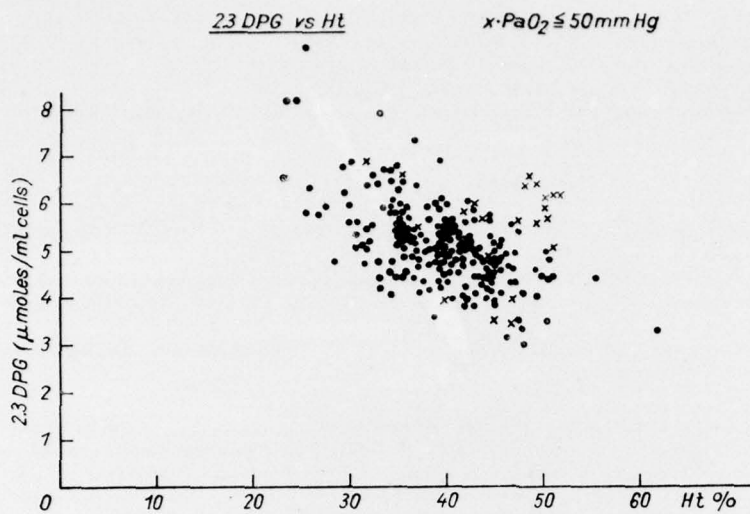


Fig. 2. The correlation between hematocrit and DPG

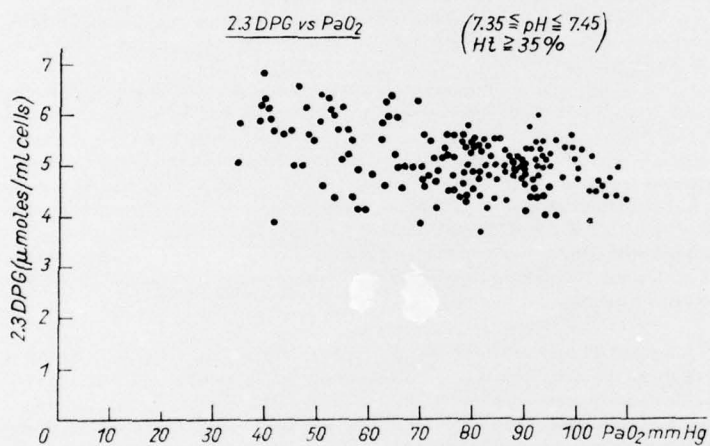


Fig. 3. The correlation between PaO_2 and DPG

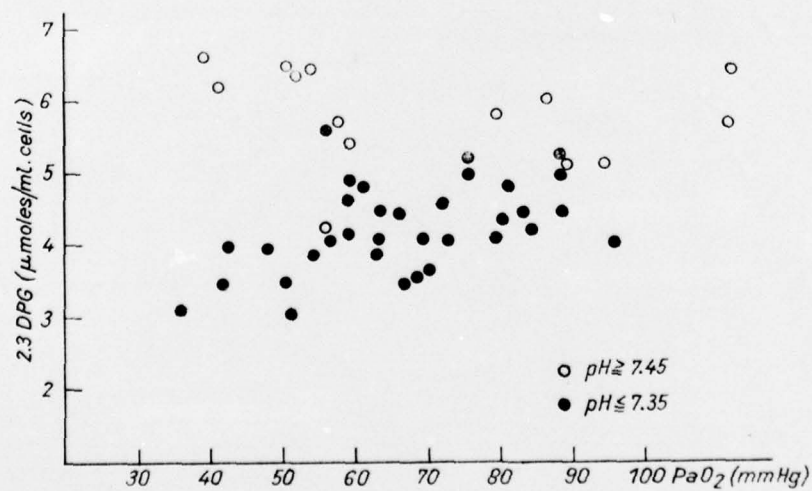


Fig. 4. The correlation between PaO_2 and DPG in acidotic and alkalotic groups

In connection with the influence of pH and PaO₂, we would like to show Figure 4, which indicates the different effect of PaO₂ in acidotic and alkalotic groups. Although the numbers of cases are not sufficient to give definite conclusions, we would like to suggest that in an alkalotic group, the correlation is negative, while the tendency is observed to have a positive correlation between DPG and PaO₂ in an acidotic group. If this tendency is definite, the explanation may be that in acidotic cases, the oxygen affinity curve is shifted to the right so much by Bohr effect, that compensation to shift back the curve to proper position is working in hypoxic state to transport more oxygen, by decreasing DPG level.

Mean and standard deviation of DPG in each group is shown in Table 1.

Table 1
Dependence of Red Cell DPG on pH, PaO₂ and Hematocrit
Physiological (pH 7.35–7.45, PaO₂ ≥ 80 mm Hg and Ht ≥ 35%)
n = 50 4.89 ± 0.46 (μmol/ml cell)

Dependence on pH (other conditions physiological)

—7.30	n = 19	4.13 ± 0.79
7.31–7.35	25	4.47 ± 0.55
7.36–7.40	105	5.29 ± 0.59
7.41–7.45	54	5.35 ± 0.56
7.46—	18	5.89 ± 0.80

Dependence on Ht (other conditions physiological)

—30	10	6.58 ± 1.18
31–35	34	5.58 ± 0.82
36–40	50	5.17 ± 0.65
41–45	46	4.86 ± 0.64
46—	19	4.80 ± 0.96

Dependence on PaO₂ (other conditions physiological)

—50	19	5.68 ± 0.67
50–70	31	5.40 ± 0.73
70–90	63	4.99 ± 0.47
90—	49	4.93 ± 0.47

DE VERDIER, Uppsala:

DPG depletion in the erythrocytes means increased oxygen affinity and a sufficient amount of oxygen can be delivered either by increasing the blood flow — as you have shown — or by decreasing the venous P_{O₂} — as some other investigators have shown. Almost all oxygen consuming enzymes in the tissues have extremely low K_M-values for oxygen. Therefore they ought to function well also at very low oxygen tensions. You have reported the interesting finding that an increase in DPG will decrease the secretion of erythropoietin. It could then be suggested that the main function of DPG is not to increase oxygen tension in the tissues, but to shut off the erythropoietin production. A prolonged erythropoietin production could lead to a too high hemoglobin production as there is a considerable lag time between the increase of the erythropoietin production and the response on the hemoglobin concentration in the circulating blood. Would you care to comment on this suggestion which I know you have considered?

VALERI, Chelsea:

I agree with Dr. DE VERDIER that red cell 2,3 DPG influences the red cell affinity *in vivo* and that the *in vivo* affinity state of red cells is related to erythropoietin production. The effect to red cell 2,3 DPG on red cell affinity *in vivo* and erythropoietin production is a slow adjustment in the oxygen transport system. Unlike Dr. DE VERDIER, I believe that red cell 2,3 DPG may be very important in the transfusion of seriously ill patients. Our laboratory has reported on the preservation of human red cells with 1–1/2 to 2 times normal 2,3 DPG using liquid and freezing procedures, and of red cells rejuvenated with solutions containing pyruvate, inosine, glucose, phosphate, and adenine. After washing, rejuvenated red cells have good posttransfusion survival and decreased affinity for oxygen. Preserved red cells with 1–1/2 to 2 times normal 2,3 DPG levels may be important in patients with compromised cardiac and cerebral circulation. Our laboratory is now studying the transfusion of red cells with low, normal, and 1–1/2 to 2 times normal 2,3 DPG levels to determine their effects on myocardial function in patients undergoing extracorporeal circulation. Red cells with increased 2,3 DPG (1–1/2 to 2 times normal) will usually circulate for 24 hours to 3 days with decreased affinity for oxygen, and during this period may reduce the heart and blood flow requirements for maintaining normal oxygen transport.

VERSMOLD, München:

Discussing the direct effect of low blood O₂ affinity it had been assumed, that an impairment of the microsomal O₂ consuming system (mixed function oxidation) might occur at a higher critical P_{O₂} than of the mitochondrial respiratory chain. In the model of the Hb-free perfused rat liver the redox state of the microsomal cytochromes P-450 and b₅ as well as of the mitochondrial cytochromes b, c, aa₃ has been directly registered by transmittance spectroscopy (Brauser 1968).

With decreasing P_{O₂} the reduction of microsomal and mitochondrial cytochromes occurred simultaneously, indicating that in the intact organ there is no significant difference in O₂ sensitivity of these systems.